

**Amendments to the Specification:**

Please replace paragraph beginning on page 1, line 7, with the following rewritten paragraph:

The present invention concerns an interference microscope and a method for operating an interference microscope. A  $4\pi$  microscope, standing wave field microscope, or an  $I^2M$  (Image Interference Microscopy),  $I^3M$  (Incoherent Interference Illumination Microscopy), or  $I^5M$  (Image Interference Microscopy and Incoherent Interference Illumination Microscopy combination) microscope is provided, in particular, as the interference microscope. At least one specimen support unit is associated with the specimen.

Please delete the paragraphs from the paragraph beginning on page 4, line 27, through and including the paragraph beginning on page 11, line 18.

On page 11, before line 24, please insert the following new paragraphs:

--The present invention also provides a method for operating a microscope with at least one objective comprising the steps of:

- providing at least one specimen support unit associated with a specimen,
- positioning the specimen together with the specimen support unit in such a way that a planar area of the specimen support unit is located in the focus region of an objective of the interference microscope, and
- determining the illumination state in a specimen region of the interference microscope on the basis of at least one planar area of the specimen support unit.

In particular, the light reflected and/or induced at the planar area is detected. For that purpose, an intensity signal profile is detected as a function of the axial position of the planar area. For detection of the axial intensity signal profile, the specimen together with the specimen support unit is moved along the optical axis of the objective or objectives, and the light reflected and/or induced by the planar area is detected in that context using a detector. Axial positioning of the specimen together with the specimen support unit could be accomplished continuously or in steps. For precise accomplishment of the signal detection, to begin with the specimen together with the specimen support unit is positioned in such a way that the planar area of the specimen support unit is located in the focus region of the objective of the interference microscope. As a result, it is generally possible to detect a signal of the light

reflected and/or induced at the planar area.--.

On page 12, before line 20, please insert the following new paragraphs:

-- A planar area of the specimen support unit configured to be detectable by light microscopy could be implemented by way of an at least partially reflective coating of a surface of the specimen support unit, for example in the form of a cover slip coated on one side. As an alternative thereto, the specimen support unit could comprise a reflective or luminescent layer between two glass plates, so that a planar area configured to be detectable by light microscopy is created by said layer. Two glass plates of differing material properties in direct contact with one another could also form a planar area configured to be detectable by light microscopy, for example if the refractive indices of the two glass plates differ, the planar area being detectable by light microscopy by way of the refractive index transition. In addition, the use of crystal or glass plates having holographic coatings or configurations can result in a planar area configured to be detectable by light microscopy. As an alternative thereto, a surface of the specimen support unit could be coated with a fluorescent layer, so that fluorescent light can be induced at said surface. Although a planar area usually has a two-dimensional extension, in this context a "planar area" is certainly also to be understood as a layer or an object having a three-dimensional extension, although also having only a small physical extension in one dimension.

A combination of these possibilities is also conceivable; in that case both a reflective and a fluorescent layer are provided, so that the fluorescent layer is excited to fluoresce by the illuminating light (i.e. fluorescent light is induced), and the illuminating light is reflected from the reflective layer.

This induced and/or reflected light is detected by a detector. Based on the detected signals, conclusions can be drawn as to the phase position directly in the specimen region of the interference microscope, as a result of which the interference microscope can be correspondingly aligned. In particularly advantageous fashion, this procedure for determining the illumination state in the specimen region of the interference microscope makes possible a reproducible and at the same time objective measurement, since the result of said measurement depends only on the surface prepared in defined fashion or on the defined properties of the planar area, and the measurement does not need to be performed on the specimen to be measured. This procedure moreover necessarily results in a reproducible

result, which is not always possible with the procedure known from the existing art in which the illumination state in the specimen region is detected at the specimen itself) since, for example, the specimen may not comprise suitable structures on the basis of which conclusions can be drawn as to the actually existing phase position of the illuminating light.

At least one planar area of the specimen support unit could be of partially reflective configuration. For that purpose, the surface could be coated. In particular, the surface could be coated in such a way that it possesses a defined degree of reflection that preferably is constant over the entire surface. The coating of the surface could be wavelength-dependent so that, for example, only light of a specific wavelength is reflected at the surface coating. A metallic or dielectric coating is provided as the surface coating; a dielectric or metallic/dielectric hybrid coating would also be conceivable.

In further advantageous fashion, at least one surface of the specimen support unit comprises at least one layer that can be excited to luminesce, in particular to fluoresce. This luminescent layer could be a monolayer. Monolayers possess a defined thickness that is determined by the dimension of the luminescent molecules used and by their arrangement on the surface. A monolayer thus represents an ideal planar structure suitable for luminescence.

In particularly advantageous fashion, the surface of the specimen support unit is equipped with several luminescent layers, each of which has different luminescent properties. These luminescent layers can be selectively excited to luminesce by light of different wavelengths, and the luminescent light emitted by the luminescent layers (which also differs in wavelength) can be selectively detected. In a preferred embodiment, several monolayers of differing luminescent properties that can be excited to luminesce are provided as the surface coating. The luminescent layer or layers can be excited to luminesce with light of a light source. This could be the light source of the interference microscope; the use of an additional light source that only excites the luminescence of the luminescent layer is also conceivable. Ideally, the light source emits light of different wavelengths, so that a surface coated with several different luminescent layers can be excited to luminesce with light of that one light source. Concretely, this could be an argon-krypton laser that simultaneously emits light of the wavelengths 488 nm, 568 nm, and 647 nm. The use of a mercury arc lamp (HBO) lamp is also possible; with this light of different wavelengths it is also possible to excite different luminescent wavelengths to luminesce.

In an alternative embodiment, provision is made for inducing light by means of non-linear processes at a planar area of the specimen support unit. In particular, coherent anti-Stokes Raman scattering (CARS) is provided as the non-linear process. CARS is a four-wave mixed process that is proportional to the square of the intensity of the light used. CARS occurs only at locations at which an optical asymmetry exists, for example a discontinuity in refractive index that is present at the surface of the specimen support unit because a refractive index transition exists there from glass to the immersion medium surrounding the specimen.

In a preferred embodiment, the light reflected and/or induced at the planar area is detected using the detector of the interference microscope. This is advantageous in particular when the light reflected/induced at the planar area lies approximately in the same power level range and wavelength range as the light of the specimen to be detected, and is adapted to the detection range of the detector of the interference microscope. It is also conceivable, however, for the light reflected/induced at the planar area to be detectable with an additional detector. For that purpose, the reflected/induced light is, by means of at least one optical component, switched out of the detected or illuminating beam path of the interference microscope and conveyed to the additional detector. For this purpose, a conventional glass plate having a defined degree of reflection/transmission could be used as the optical component. A dichroic beam splitter, a filter, a prism, a grating, and/or a spectrally sensitive arrangement would also be conceivable as the optical component for switching out the reflected/induced light. In particular when the light induced at the planar area of the specimen support unit is fluorescent light of a fluorescent layer, said fluorescent light can be conveyed to the detector in spectrally selective fashion using a spectrally sensitive arrangement. The spectrally sensitive arrangement could comprise, for example, lenses, stops, and a prism or a grating.

Detection of the light reflected and/or induced at the planar area of the specimen support unit could be accomplished in widefield mode. The widefield mode involves planar illumination and/or detection, such as is present, for example, in a standing wave field microscope or an I<sup>2</sup>M microscope. The detector detecting the light reflected/induced at the planar area could correspondingly be embodied as a planar detector, for example in the form of a CCD chip.

The light reflected and/or induced at the planar area of the specimen support unit could be detected confocally. In that case a confocal illumination is provided; i.e. the light serving for

illumination is focused onto a point of the focal plane of the microscope objective. For confocal detection, there is arranged in front of the detector a pinhole that preferably is arranged in a plane corresponding to the specimen plane of the objective. The illumination or detection pinhole of the interference microscope could be provided as the pinhole. If detection of the light reflected/induced at the planar area of the specimen support unit is accomplished using the confocal detector of the interference microscope, the pinhole arranged in front of the detector is the detection pinhole of the interference microscope. If the illumination pinhole serves as the pinhole, an optical component arranged between the light source and the illumination pinhole could switch the light reflected/induced at the planar area of the specimen support unit out of the illumination beam path and convey it to a correspondingly arranged detector.

In a preferred embodiment, provision is made for the determination of the illumination state in the specimen region of the interference microscope to be performed using light of at least one additional light source. As already mentioned, this can be a laser system, a laser, or an HBO lamp.

In a concrete embodiment, the specimen support unit is fabricated of glass. The surfaces of the specimen support unit ideally has a high degree of surface planarity, which is also exhibited by the coating or fluorescent layer that may be applied onto a surface. Concretely, the specimen support unit could be configured as a cover glass. These could be commercially available cover glasses. In a particularly preferred embodiment, the specimen is arranged between two specimen support units, preferably between two specimen support units configured as cover glasses. Preferably the planar area of the specimen support unit that faces toward the specimen is configured in reflectable or inducible fashion.

In particular, provision is made for detecting several axial intensity profiles, specifically at one and/or several points of the focal plane. In a double confocal scanning microscope or 4 $\pi$  microscope, the various points at which one or more intensity signal profiles are respectively to be detected are arrived at by means of a beam scan. As a result, it would also advantageously be possible to ensure that a defined phase relationship exists for several points of the focal plane, so that the specimen can be illuminated and detected with the beam scanning method, the same defined phase relationship existing for different beam deflection angles and scanning angles. The essential advantage of the beam scanning method lies in

rapid specimen detection. An alternative to the beam scanning method would be a specimen scanning method, in which the specimen is moved, for example in meander fashion, through the focus of the stationary illuminating beam.

Particularly with regard to a change in the state of the interference microscope, provision is made for several detections of axial intensity signal profiles to be performed, for example in order to determine the illumination state in the specimen region of the interference microscope at different times. Provision is preferably made for detections of the intensity signal profile also to be made during a specimen detection. If light of different wavelengths is used for determination of the illumination state in the specimen region of the interference microscope, provision is made for a detection of an axial intensity signal profile to be made in each case for the light of each wavelength. Each light of a different wavelength reflected/induced at the planar area would correspondingly be associated with a detector and detected by the latter. In this case a simultaneous detection of the light of the different wavelengths would be possible. It would also be conceivable to convey the light of different wavelengths to one and the same detector each time; in this case only sequential detection of the light of the individual wavelengths is possible.

In a further method step, provision is made for the detected axial intensity signal profile to be evaluated using an algorithm. Said algorithm serves principally for determination of the phase relationship of the illuminating or detected light present in the specimen region of the interference microscope.

Concretely, provision is made for the algorithm first to determine the center point of the axial intensity signal profile. In addition, the height of the signal at the center point of the intensity signal profile is determined.

Additionally or alternatively, the algorithm could also compare the signal points of two points equidistant from the center point of the intensity signal profile. The points equidistant from the center point could, for example, be accomplished at the location at which, in  $4\pi$  microscopy, the secondary maxima or the two first minima are usually arranged. Provision could furthermore be made for the algorithm to evaluate the symmetry of the intensity signal profile with respect to its center point.

Lastly, provision is made for the interference microscope to be aligned as a function of the

illumination state in the specimen region. Alignment of the interference microscope is performed with the goal of implementing constructive interference in the illumination focus. A corresponding control system could be provided for that purpose. Concretely, the alignment could encompass an optical path length change of an interferometer beam path segment. This could be implemented, for example, by parallel displacement of a corresponding mirror.

The detection and alignment operations described above are repeated and are coordinated with the drift behavior of the interference microscope. For example, if the interference microscope is subject to relatively severe temperature fluctuation, frequent repetition of the detection and alignment operations will be necessary in order to control the illumination state in the illumination focus in such a way that almost exclusively constructive interference is present.--.